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Frequent loss of chromosome 14 in atypical and malignant meningioma: identification of a putative 'tumor progression' locus

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Formation of meningiomas has been associated with the loss of genetic material on chromosome 22. To approach the additional chromosomal events that underlie progression of these tumors to malignancy, we have examined several other chromosomal regions for loss of heterozygosity (LOH) in these tumors. Fifty-eight tumors, comprising 43 benign meningiomas, 11 atypical meningiomas and four malignant meningiomas, were examined. While the loss of chromosome 22 was seen in approximately half of all these tumors, regardless of their malignancy, the most frequent chromosomal losses observed in the malignant and atypical tumors were on the long arm of chromosome 14. Thirty-nine tumors were informative for at least one of the three markers on chromosome 14 that we tested. Of these, 7/14 malignant and atypical tumors showed LOH in contrast to only 1/25 benign tumors. Other loci that showed LOH in malignant tumors, although at a much lower frequency, were on chromosomes 17p and 1p. The high frequency of LOH for loci on chromosome 14q in atypical and malignant tumors suggests the presence of a tumor progression gene at this locus. In one of the malignant meningiomas heterozygosity was lost at *D14S13* and *D14S16* but retained at the proximal marker *D14S43* as well as the more distal marker *D14S23*. This suggests that an interstitial deletion occurred in this tumor which should be useful for further refining the position of the putative tumor progression locus.

Keywords: loss of heterozygosity; chromosome 14; meningioma

Introduction

Meningiomas comprise approximately 15% of all intracranial neoplasms and 25% of spinal cord tumors. They are thus one of the most common tumors of adults, with the age of incidence between 20 and 60 (Russell and Rubinstein, 1990). These tumors generally occur as solitary tumors but multiple meningiomas have been described. They are thought to arise from the cells forming the outer layer of the arachnoid membrane, related cells such as arachnoidal fibroblasts, or undifferentiated precursor cells of the meninges termed meningioblasts (Challa and Markesbery, 1992; Russell and Rubinstein, 1990). Their pathology is subject to considerable variation, thus giving rise to controversies over the histogenesis and terminology of these tumors. Most meningiomas are slow growing, encapsulated tumors and are therefore often termed 'benign'. Histologically they are characterized by a whorled pattern of cells and calcified structures known as psammoma bodies. A small fraction of meningiomas progress into a more aggressive, malignant form that can be distinguished from the 'benign' meningioma by the absence of cellular architecture (referred to as 'sheeting'), the presence of excessive mitotic activity, necrosis and invasion of neighboring tissue. Meningiomas that exhibit histological features that are in between those of 'benign' and malignant meningiomas pose the most difficulty in classification as well as prognosis. These tumors are often referred to as 'atypical' meningiomas.

The first clues to finding genetic loci possibly involved in tumor initiation or progression in meningiomas and schwannomas were provided by cytogenetic studies that demonstrated loss of chromosome 22 as the most common chromosomal abnormality in these tumors (Zankl and Zang, 1972; Casalone *et al*., 1990). Other investigators examined loci on chromosome 22 with polymorphic DNA markers and established that LOH on this chromosome was a common pathogenic mechanism for tumor formation in meningiomas as well as acoustic neuromas (Seizinger *et al*., 1986, 1987; Dumanski *et al*., 1987).

To determine which additional chromosomal events were involved in the formation and/or progression of meningiomas we examined cytogenetic material from meningiomas to identify chromosomes that were lost or rearranged. The examination of cytogenetic material from meningiomas also suggested loss of chromosomes other than 22, indicating that other chromosomal regions might play a role in tumor formation and progression. We have examined VNTR markers on chromosomes 1, 2, 4, 9, 10, 14, 17, 19, 20 and 22. In benign meningiomas LOH was seen on chromosome 22 (eight of 25 informative cases), chromosome 10 (three of 28 informative cases), chromosome 14 (one of 25 informative cases), and chromosome 19 (one of four informative cases) suggesting a chromosomal location for other genes involved in tumor formation. Our examination showed a significant number of cases in which loss of chromosome 14 was observed.

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Results

All tumors were graded by histopathological examination. Benign meningioma is characterized by the presence of whorls, ill defined cell borders, pale or sometimes optically empty intranuclear vacuoles, and psammoma bodies. These tumors generally do not show extensive mitoses, prominent nucleoli, or nuclear pleomorphism. Malignant meningioma is characterized by the presence of necrosis, increased cellularity, sheeting and nuclear pleomorphism, presence of mitoses, and invasion of surrounding tissue. Atypical meningioma comprises the group of meningiomas that show characteristics that are intermediate between benign and malignant such as moderate nuclear atypia with occasional prominent nucleoli, rare foci of necrosis, and scattered mitoses.

Genomic DNA from matched tumor and blood samples was digested with appropriate restriction enzymes, fractionated on agarose gels, and blotted onto nylon membranes. Blots were hybridized with radiolabeled probes. The chromosomal location of probes was confirmed using *in situ* hybridization to chromosome preparations. The probes CMM101 and CKKA39 both displayed hybridization signals on distal chromosome 14q. No signal was detected for THH37 by *in situ* hybridization, therefore it was mapped using a panel of somatic cell hybrids by Southern blotting.

All tumors were first tested for loss of chromosome 22. LOH on chromosome 22 was seen in roughly 30% (12 of 39) of the meningiomas regardless of the malignancy grade of the tumor, consistent with previous observations (Katsuyama *et al*., 1986; Seizinger *et al*., 1987; Casartelli *et al*., 1989). The observation of chromosome 14 loss in karyotypes of meningiomas (Katsuyama *et al*., 1986; Casartelli *et al*., 1989) prompted us to use the highly polymorphic DNA markers CMM101 (*D14S13*), THH37 (*D14S16*) and CKKA39 (*D14S23*) to study LOH in these tumors. A total of 58 meningiomas was examined for LOH using matched DNA samples from tumor and peripheral lymphocytes. Based on the results of these matched DNA samples, 39 were informative with probes for at least three loci (Table 1). The set of informative tumors included in this study comprised 25 'benign', 10 atypical and four malignant meningiomas. In order to determine whether additional deletion events occurred we also looked for LOH on 10 other chromosomal loci with the following polymorphic DNA markers: MLAJ1 (*D1S61*), MCT106 (*D2S61*), pYNZ32 (*D4S23*), DOSL5 (*D9S1*), EFD75 (*D10S25*), p5-1 (*D10S1*), pYNH 37.3 (*D17S28*), EFD4.2 (*D19S22*), CMM6 (*D20S19*) and NB129 (*D22S193*). Representative samples of the data obtained in five matched tumor/blood pairs with probes on chromosome 14 and 22 are shown in Figure 1, and the summarized data on all 39 tumors are presented in Table 1.

Of the 'benign' tumors studied, LOH on chromosome 14 was seen in one of 25 informative events. Other chromosomal loci showing LOH were *D9S1* (one of 14) and *D19S22* (one of 12 informative cases). In contrast, four of 10 atypical meningiomas and three of four malignant meningiomas showed LOH for markers on chromosome 14. The other chromosomal loci lost in the higher grade tumors included *D1S61* on chromosome 1p (two of seven informative cases) and *D17S28* (two of seven informative cases). In all cases where markers on both chromosome 14 and chromosome 22 were heterozygous, LOH on chromosome 14 was paralleled by LOH on chromosome 22 (Figure 1, cases 1 and 4). Overall, approximately 40% (six of 14) of the higher grade (atypical and malignant) meningiomas showed LOH on chromosome 14, whereas only one of 25 'benign' meningiomas showed loss of these chromosome 14 markers. Three atypical/malignant tumors (cases 2, 3, and 5, Table 1) did not exhibit LOH for chromosome 22 but did exhibit LOH for chromosome 14. This is not unexpected since LOH for chromosome 22 is not required for tumor formation in the majority of meningiomas (Katsuyama *et al*., 1986); Seizinger *et al*., 1987; Casartelli *et al*., 1989). Alternatively, the apparent retention of homozygosity could be explained if these loci were hemizygous in these cases.

One of the atypical meningiomas studied (Figure 2, Case 6 and Figure 3) showed LOH on chromosome 14 with the markers *D14S13* and *D14S16*, but apparently

Table 1 LOH for markers on chromosomes 14 and 22.

Case	*Tumor grade*	*CMM101* (D14S13)	*THH37* (D14S16)	*CKKA39* (D14S23)	*Chromosome 22*
1	malignant	–,**2**	**1**,–	**1**,–	–,**2**
2	malignant	–,**2**	**1**,–	–,**2**	U
3	malignant	–,**2**	**1**,–	–,**2**	1,1
4	atypical	–,**2**	**1**,–	**1**,–	**1**,–
5	atypical	–,**2**	U	**1**,–	U
6	atypical	**1**,–	1,2	**1**,–	**1**,–
7	malignant	1,2	1,2	1,2	N
8	atypical	**1**,–	**1**,–	**1**,–	**1**,–
9	atypical	N	1,2	1,2	1,2
10	atypical	U	N	U	N
11	atypical	U	N	U	1,2
12	atypical	U	N	N	N
13	atypical	U	U	1,2	N
14	atypical	1,2	U	1,2	1,2
15	'benign'	1,2	U	1,2	**1,–**
16	'benign'	1,2	1,2	1,2	N
17	'benign'	1,2	1,2	1,2	N
18	'benign'	U	1,2	U	1,2
19	'benign'	U	1,2	U	N
20	'benign'	1,2	N	1,2	N
21	'benign'	1,2	U	N	1,2
22	'benign'	1,2	1,2	N	1,2
23	'benign'	1,2	1,2	N	**1**,–
24	'benign'	1,2	N	1,2	N
25	'benign'	1,2	1,1	N	1,2
26	'benign'	N	1,1	1,2	–,**2**
27	'benign'	N	1,2	1,2	N
28	'benign'	U	1,2	1,2	N
29	'benign'	1,2	U	1,2	1,2
30	'benign'	1,2	U	U	–,**2**
31	'benign'	U	1,2	1,2	**1**,–
32	'benign'	1,2	1,2	1,2	N
33	'benign'	N	1,2	1,2	1,2
34	'benign'	N	1,2	1,2	1,2
35	'benign'	**1**,–	1,2	1,2	N
36	'benign'	1,2	1,2	1,2	**1**,–
37	'benign'	N	U	1,2	**1**,–
38	'benign'	1,2	U	1,2	N
39	'benign'	N	U	1,2	**1**,–

Alleles detected by each probe are designated 1 (larger sized allele) or 2 (smaller sized allele), allele loss is denoted by –. The allelotype of tumors showing LOH is indicated in **bold face**. Cases where the probe could not distinguish between allele loss and allele homozygosity are marked 'U' for uninformative, and 'N' denotes not available. LOH on chromosome 22 was determined by combining the results for probes at the loci *D22S1*, *D22S29*, *D22S56* and *D22S193*

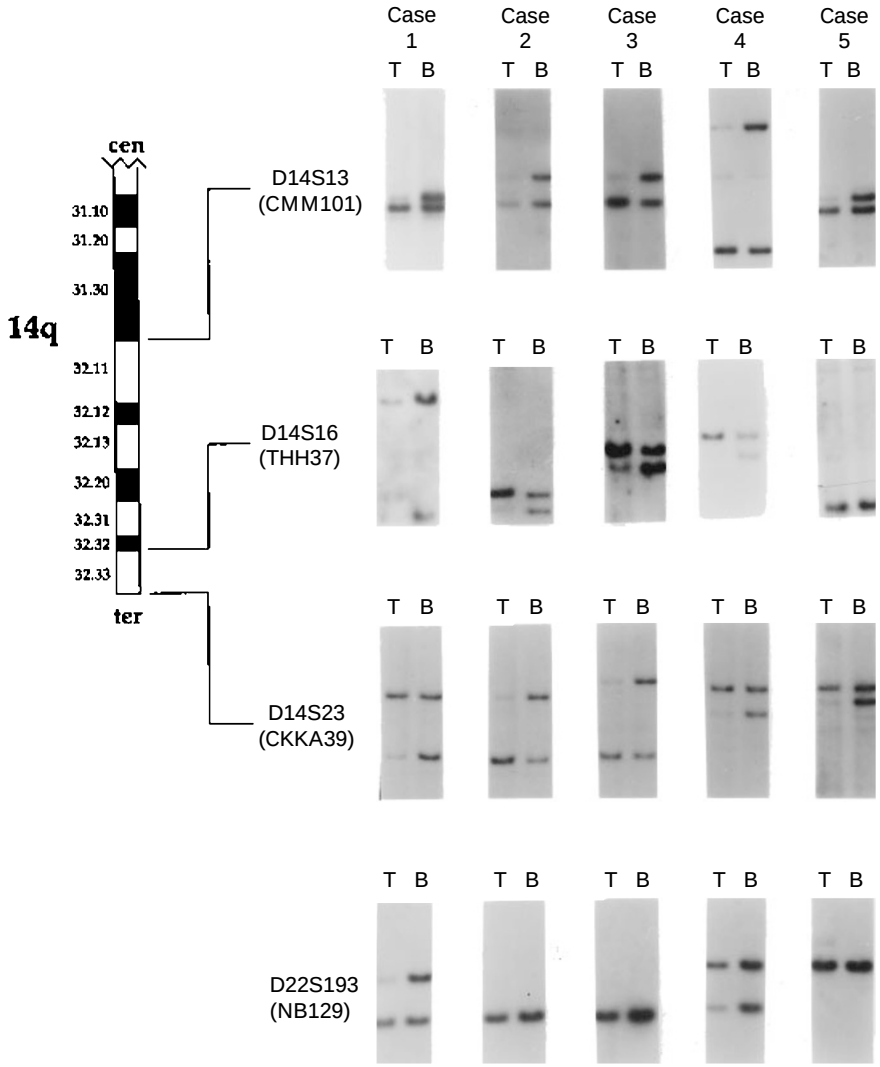

Figure 1 Representative cases of atypical or malignant meningiomas in which LOH was observed with the markers CMM101 (*D14S13*), THH37 (*D14S16*) and CKKA39 (*D14S23*). The order of these markers on the long arm of chromosome 14 is based on the genetic linkage map of Nakamura *et al*. (1989). Also shown (bottom panel) is an analysis of tumor and blood DNA from all five cases using the chromosome 22 probe NB129 (*D22S193*). The faint residual signal is from blood and stroma in the tumor sample

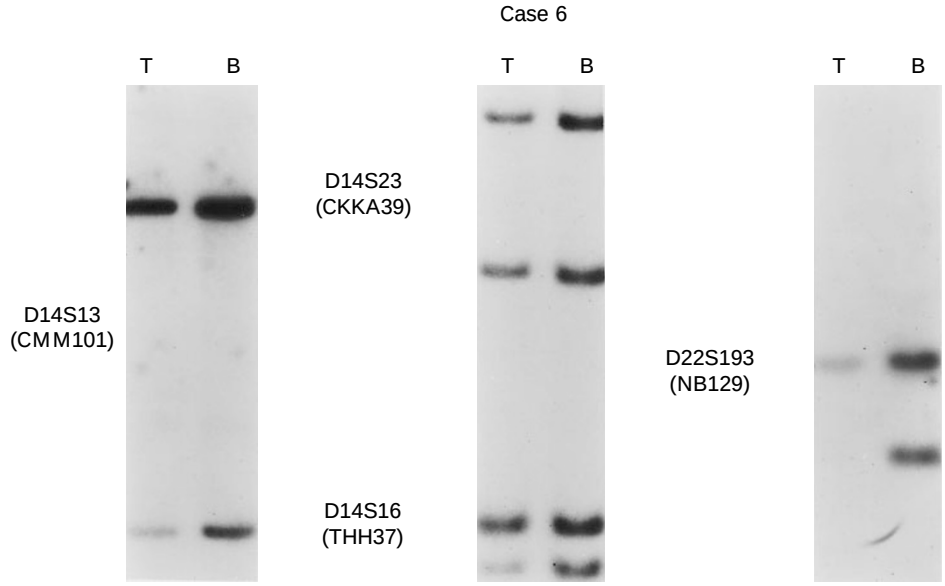

Figure 2 Fine mapping of deletion on chromosome 14q. LOH was observed in a meningioma (Case 6) with the proximal markers CMM101 (*D14S13*) and THH37 (*D14S16*) but heterozygosity was retained at the distal marker CKKA39 (*D14S23*). LOH on chromosome 22 was observed in this patient using the marker NB129 (*D22S193*). Lanes containing tumor DNA are marked 'T' and lanes containing matched control (blood) DNA are marked 'B'. The faint residual signal is from blood and stroma in the tumor sample

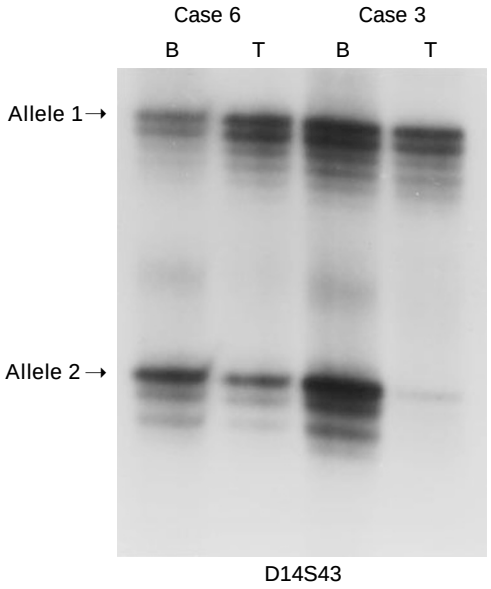

Figure 3 PCR of the locus *D14S43* containing a dinucleotide polymorphism (GT) was used to determine the extent of the interstitial deletion in Case 6. A tumor which showed loss of the entire chromosome 14 by cytogenetic analysis was used as a control (case 3). Lanes containing tumor DNA are marked 'T', lanes containing matched control (blood) DNA are marked 'B'

retained heterozygosity at the locus *D14S23* (the closest flanking locus on the telomeric side) suggesting the presence of an interstitial deletion on one copy of chromosome 14 in this tumor. While the extent of the deletion was clearly defined on the telomeric end by *D14S23*, it was difficult to precisely define the boundary at the centromeric end. Several probes that have been placed centromeric to *D14S13*, such as pMZ9 (*D14S18*), or in the immediate vicinity of this locus, such as pCMM62 (*D14S21*), were uninformative in this tumor. The closest available informative marker on the centromeric side of the deletion was at the locus *D14S43*. This marker detected a dinucleotide polymorphism at *D14S43* and has been described by Sharma *et al.* (1991). The order of these markers on the long arm of chromosome 14 has been determined to be cen-*D14S43*-*D14S13*-*D14S16*-*D14S23*-tel (Nakamura *et al.*, 1989; Hofker *et al.*, 1990; NIH/CEPH Collaborative Mapping Group, 1992). Consistent with our previous observations in malignant meningioma, this tumor also showed LOH of markers on chromosome 22 suggesting that the loss of the locus on chromosome 14 plays a role in the pathway towards malignancy rather than be the primary event in tumor initiation.

Discussion

The availability of highly polymorphic DNA markers and their use in LOH studies in tumors has allowed the identification of many of the individual events that go into the formation of tumors and their progression towards malignancy (Marshall, 1991). This approach has allowed the identification of deletions on chromosome 22 as being the most common chromosomal event in the formation of meningiomas. The additional steps that are involved in the progression of these tumors from the 'benign' to atypical and finally malignant stage have been the subject of this study. Of the polymorphic markers on 10 different chromosomes that were examined in high grade meningiomas (atypical and malignant tumors) the most frequent chromosomal losses were on chromosome 14 using the markers *D14S13*, *D14S16* and *D14S23*. The observation of loss of this chromosome in meningiomas has been previously documented in studies of tumor karyotype, however many of these studies made the distinction only between 'benign' (those that did not show frank invasion) and malignant meningiomas (those that showed frank invasion). The existence of a class of tumors, referred to as atypical meningiomas, that possess features intermediate between benign and malignant meningiomas poses a problem in the classification of their grade causing them to generally be subsumed under the class of benign tumors. Our observation of chromosome 14 loss in atypical and malignant meningiomas would suggest that atypical meningiomas constitute a separate class, distinct from benign tumors. It will clearly be important to extend this study to see whether LOH on chromosome 14 in atypical meningiomas is a prognosis of future malignancy in these tumors.

The most frequently described chromosomal aberration of chromosome 14 in solid tumors is the loss of the entire chromosome. Loss of chromosome 14 has been described in meningioma (Katsuyama *et al.*, 1986; Al Saadi *et al.*, 1987), neuroblastoma (Suzuki *et al.*, 1989), Ewing's Sarcoma (Turc-Carel *et al.*, 1988) and Leiomyosarcoma (Boghosian *et al.*, 1989). Furthermore, the description of chromosomal translocations that involve the same cytogenetic band on chromosome 14 suggest that a common genetic locus might be involved in the pathway towards tumorigenesis (Katsuyama *et al.*, 1986; Al Saadi *et al.*, 1987; Seizinger *et al.*, 1987) as well as in other solid tumors such as neuroblastoma (Suzuki *et al.*, 1989; Srivatsan *et al.*, 1991), breast tumors (Gebhart *et al.*, 1986; Bello and Rey, 1989), ovarian tumors (Atkin *et al.*, 1990), astrocytoma (Bigner *et al.*, 1986), retinoblastoma (Chaum *et al.*, 1984) and malignant melanoma (Pedersen and Wang, 1989). One malignant meningioma was identified which showed loss of heterozygosity for the markers *D14S13* (pCMM101) and *D14S16* (THH37) but retention of heterozygosity for the proximal flanking marker *D14S23* or the distal flanking marker *D14S23*. This further allows the placement of the putative tumor progression locus between *D14S43* and *D14S23* (assuming there were no terminal translocations in this tumor), a region encompassing the distal third of chromosome 14q. The cytogenetic placement of this locus is therefore between 14q24.3 and 14q32.3 (Nakamura *et al.*, 1989; Sharma *et al.*, 1991; NIH/CEPH Collaborative Mapping Group, 1992), a region that contains the genes ELK-2, a member of the ETS family of oncogenes (Rao *et al.*, 1989), AKT, a gene showing amplification in gastric adenocarcinomas (Staal, 1987), and the oncogene TCI-1 (Croce *et al.*, 1985). These genes are generally classified as 'proto-oncogenes' and are thought to play a role in tumor formation when they are activated by mutation or translocation (Croce *et al.*, 1985; Staal, 1987; Rao *et al.*, 1989). It is not clear whether these genes can also act as tumor

suppressors in other cell types. Experiments to test the expression of these genes in meningiomas might yield information on the role of these genes in tumor formation and progression.

While the loss of chromosomal material on the long arm of chromosome 14 clearly indicates the presence of a tumor suppressor gene in this region, the apparent association of a higher grade of malignancy in meningiomas with loss of chromosome 14 in this study requires further study on a larger number of atypical and malignant meningiomas. It is possible that chromosome 14q24.3-32.3 harbors one of the genetic loci involved in the progression of meningiomas to malignancy in a manner similar to the multi-step mechanisms described in other cancers (Fearon and Vogelstein, 1990).

Materials and methods

DNA was extracted from tumors and corresponding normal tissue (peripheral blood). Tumor specimens were frozen on dry ice or liquid nitrogen immediately after surgery and were ground in a mortar to the consistency of a coarse powder. 0.5 g of this powder was resuspended in TNE-SDS-Proteinase K (10 mM Tris-HCl pH 8.0, 100 mM NaCl, 1 mM EDTA, 1% SDS, 200 μg/ml Proteinase K) and incubated overnight at 50°C. DNA was isolated from peripheral blood lymphocytes by homogenizing peripheral venous blood in a solution containing 0.64 M sucrose, 20 mM Tris, 10 mM Magnesium chloride, 5% (v/v) Triton-X-100 pH 7.6 and then resuspending in TNE-SDS-Proteinase K overnight at 50°C. DNA was purified from these samples by phenol/chloroform extraction, precipitated with ethanol, and resuspended in TE (10 mM Tris pH 8.0, 1 mM EDTA). This DNA was digested with TaqI, PstI, or BglI (New England Biolabs) using conditions recommended by the manufacturer. The resulting DNA fragments were fractionated by agarose gel electrophoresis and transferred to Hybond N^+ membranes (Amersham) using the method of Southern (1975). Southern blots were hybridized with probes for several polymorphic markers. The probe NB129 (*D22S193*) was a kind gift from Dr E Zwarthoff (Erasmus University, Rotterdam, The Netherlands). Probes pH35 (*D22S44*) and pH97b (*D22S56*) were kind gifts from Dr Beverly Emanuel (Children's Hospital, Philadelphia). Probes CMM101 (*D14S13*), THH37 (*D14S16*), CKKA39 (*D14S23*), MLAJ1 (*D1S61*), MCT106 (*D2S61*), pYNZ32 (*D4S23*), DOSL5 (*D9S1*), EFD75 (*D10S25*), p5-1 (*D10S1*), pYNH 37.3 (*D17S28*), EFD4.2 (*D19S22*), CMM6 (*D20S19*), pMS3-18 (*D22S1*), pEFZ31 (*D22S32*), and W22D (*D22S29*) are as described by the American Type Culture Collection (ATCC). A dinucleotide repeat polymorphism was used to examine LOH at the locus *D14S43*. PCR reactions were carried out using the primers 5′-TGGAACACTCAGGCGA-3′ and 5′-CCAGAGCCACTTTCTAC-3′ as described by Sharma *et al.* (1991) under the following conditions: A total volume of 25 μl containing 500 ng genomic DNA, 10 pmoles of each primer, 1.5 mM $MgCl_2$, 200 μmoles dNTP's, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 0.5 units of Taq polymerase (Cetus, Emeryville, CA) and 0.01% gelatin. Amplification was carried out for 35 cycles with denaturation at 94°C for 60 s, annealing at 55°C for 60 s, and extension at 72°C for 30 s in a thermal cycler made by MJ Devices. All probes for hybridization to Southern blots were labeled by random priming (Feinberg and Vogelstein, 1983). Approximately 100 000 c.p.m. of probe was boiled and added to 10 ml of SDS-PEG (10% Sodium Dodecyl Sulfate, 7% Polyethylene Glycol) at 65°C. This solution was added to the Hybond N^+ membranes (Amersham) and incubated overnight at 65°C. Membranes were washed at 65°C for 40 min in 0.1 × SSC and 0.1% SDS with two changes of the wash solution. Washed membranes were placed between sheets of Saran wrap and exposed to film (Kodak XAR-5) at −70°C for varying lengths of time.

Karyotypes of peripheral blood lymphocytes were obtained from short term cultures using classical cytogenetic protocols. All probes were labeled with biotin by nick translation and suppression hybridization was performed as described (Lichter *et al.*, 1988; Cremer *et al.*, 1988), with the following exceptions. Each hybridization reaction (10 μl volume) contained 300 ng of probe, 2.0 μg of human Cot-1 DNA (GIBCO BRL, Gaithersburg, MD) and 8.0 μg of salmon sperm DNA in a cocktail of 50% formamide, 10% Dextran Sulfate and 6 × SSC (pH 7.0). Probe and target DNA were denatured simultaneously under a sealed coverslip for 7–10 min at 80°C. Post hybridization washing and detection with 10 μg/ml FITC-Avidin DCS (Vector Laboratories, Burlingame, CA) were as described (Klinger *et al.*, 1992). All samples were counterstained with 100 ng/μl propidium iodide and analysed using a Zeiss Axioplan microscope equipped for epifluorescence. Single-exposure photographs were taken directly from the microscope with Kodak Gold 400 film.

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